THE
BIOLOGICAL BULLETIN

PHOTIC SIGNALING IN THE FIREFLY *PHOTINUS GREENI*

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It has been known for many years that most lampyrid fireflies use their light emission to bring the sexes together. Often the attraction is achieved by a characteristic signal system or code which enables male and female of a particular species to recognize each other even in the presence of other species. In many members of the genus *Photinus* the courtship exchange involves a flying male, flashing in a fixed rhythm, and a perched female who does not flash spontaneously but only in response to a proper signal. In some species the specificity of the code is known to depend on the female always responding at a fixed interval after seeing an acceptable signal and upon the male recognizing that interval. Within considerable ranges, color, intensity and duration of flash are not critical to the signals of either male or female, and flashes of artificial light can be substituted for either firefly in the dialogue.

Firefly codes are interesting physiologically because they afford clues to the neural control of flashing. It is known that the rhythmic flashing of the male is controlled by a pacemaker in the brain and that the timing of both the male's cycle and the response flash of the female can be influenced by light signals received through the eyes. These responses require visual processing, central nervous processing and conduction of the excitation through the main nerve trunk and peripheral nerves into the light organ and along a pathway of several steps in the luminous tissue. The minimum time occupied by this composite excitation process, the physiological "latency," as measured after stimulation of the eye by light or electricity in a number of species, is in the 150–250 msec range at 25° (Case and Buck, 1963; Buonamici and Magni, 1967; Magni, 1967; Case and Trinkle, 1968; Papi, 1969). Almost always, however, the actual behavior in nature involves far longer delays. For example, the response delay of the female of *P. pyralis* averages about 2 seconds, or about 10 times the minimum eye-to-lantern latency (Buck, 1937; Lloyd, 1966b). It is clear, therefore, that actual firefly codes are constructed by incorporating different but fixed amounts of central nervous delay into the overall perception-response circuit.

In *P. pyralis,* where both male and female emit only single flashes, there is no ambiguity about time relations within the signal code. In the *P. consanguineus*

complex, however, where the male emits flashes in pairs (McDermott, 1910, 1911, 1912, 1914, 1917; Lloyd, 1966a, 1966b, 1969) some interesting questions arise. It is clear that both flashes in the male's species "phrase" (Lloyd) are necessary for communication, since the female will only very rarely respond to a single flash, but it is not known what functions the two flashes perform.   One could imagine, for example, that the female times her response either from the first of the male's flashes (the second serving only a confirmatory or reinforcing role) or from the second (the first perhaps serving to prepare or "prime" her response system).

Apart from physiological considerations, analysis of firefly codes bears importantly on the species concept and the evolution of communicative behavior. Barber (1951), McDermott and Buck (1959) and Lloyd (1966b, 1971) have adduced considerable evidence for the behavioral separation of forms that are nearly or entirely indistinguishable by conventional morphological criteria but are presumably isolated reproductively by the specific codes.   Since this work has been based largely on visual observation it is clearly desirable to document the codes as quantitatively as possible and explore the limits of variation within the signal systems—that is, the ranges of timing, duration and shaping of the male's signal in relation to its acceptability to the female, and the extent to which the female's latency can vary without breaking contact with the male.   The *consanguineus* group is ideal for such study both because its code is relatively simple and because the species complex appears to consist of only a few members.   The group is presently divided into three species, *P. macdermotti* (Lloyd, 1966a), *P. greeni* (Lloyd, 1969) and *P. consanguineus* s.s. (Lloyd, 1966a, 1966b, 1969) on the basis mainly of apparent differences in timing within the phrases of the males and in the response intervals of the females.   However, all three species range widely over the Eastern United States and show enough local variation in behavior and morphology to confuse their interrelations.

A prime reason for attacking the above-mentioned problems by a field study prior to laboratory investigation is the well-known tendency for fireflies, particularly males, to flash abnormally in captivity.   In addition, the consistent differences sometimes found between individuals argue for the sampling of a considerable population.

MATERIAL AND METHODS

The species studied is one that is active in Woods Hole, Mass. in late July and the first three weeks of August.   Though designated *"consanguineus"* in a preliminary report (Buck and Buck, 1965) it more closely resembles the later named *P. greeni* in behavior and is provisionally so identified though differing somewhat in morphology from the type of that species, described from Florida. The Woods Hole form begins its evening activity at about 8 P.M. (EDST) when the surround is still quite light.   The flight of the male is mostly over within an hour, though responsive females can be found for an additional hour.

The experimental work concentrated on exploring the modifiability of the male's signal, substituting the light of an ordinary pushbutton flashlight or electric torch for the male in dialogues with females in the field.   However, enough males were attracted, using torch in place of female, to confirm the expected importance

in courtship of the interval between the male's second flash and the female's response flash. As is usual in such simulated courting, the beam of the torch was not pointed directly at the firefly. Rather the intensity of the signal was adjusted crudely to decreasing distance by shielding it more and more against ground vegetation. Even so, both the intensity of the light reaching the firefly's eye and the apparent area of the (reflected) source must have varied enormously.

In these exchanges the various flashes were recorded by means of a portable single-channel photometer, developed by Dr. Frank Hanson, which makes use of an RCA 1P21 photomultiplier and Cambridge Transcribe chart recorder. Since the flashes of the fireflies and the incandescent torch lamp had very similar rise times (*ca.* 50 msec), latencies and interflash intervals in the chart records were measured peak to peak. Because of variable orientations and distances of fireflies and torch vis a vis the photometer, apparent intensities of the recorded flashes are not comparable. Since flash frequency and response times are also highly dependent on temperature, absolute values have little significance *per se.*

Statistical parameters used are the standard error (indicated by "±") and the coefficient of variation "V."

RESULTS

1. *Courtship dialogue*

The female of *P. greeni,* though fully winged, does not ordinarily fly but perches on vegetation near the ground. The male flies only a meter or so above the ground, repeating a two-flash phrase about every 5 seconds (at 27°) (Fig. 1). When a male flashes within range of a female (up to 6 or 7 m depending on environmental illumination and terrain) the female replies after his second flash (Fig. 2). The male then advances toward her in a succession of such exchanges and eventually alights near her and completes the journey on foot. The interphrase interval of the male is not critical, since females will respond to an actively searching male (or torch) that leaves only three seconds or so between phrases or will remain responsive over many minutes during which no stimulus is received.

When the male of *P. greeni* breaks rhythm, as for example when abandoning a nonproductive dialogue, the interruption comes after the second flash of the phrase, not between flashes of the pair. This supports the expectations that the flashes constituting the species-specific pattern are delivered as a sequential unit, whereas the phrase-repetition rate, though usually quite regular, can be interrupted or varied to a much greater extent.

The female's flash is surprisingly bright considering that her lantern is only about a quarter of the area of the male's. Her flash is more than twice as long as that of the male (*ca.* 220 msec—Figs. 2–7). As in many other photinids the female flexes and twists her abdomen when delivering her response flash so that the surface of the lantern faces toward the male.

2. *Flash periodicity in male*

The male of *P. greeni* emits bright sharp flashes of yellowish light lasting about 100 msec. The interval between the two flashes of the phrase averaged 1.30 ± 0.01 sec in a male during 10 exchanges with a female, 1.35 ± 0.007 sec in

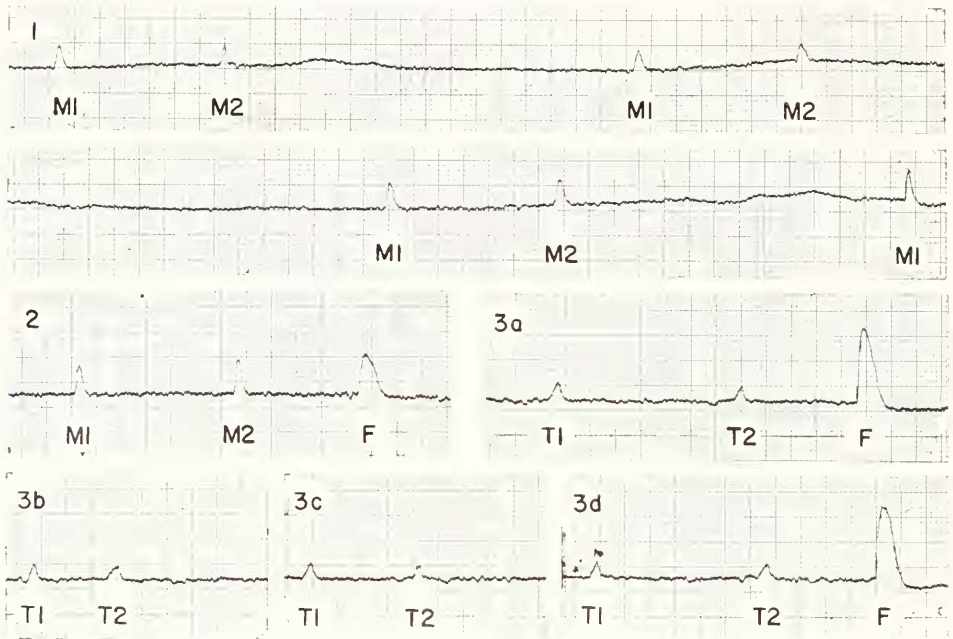FIGURE 1. Normal spontaneous flashing of male of *P. greeni* in flight while investigating torch answers. Continuous record of 3½ successive phrases; time scale in this and all photometer records = 200 msec between heavy vertical lines.

FIGURE 2. One signal-answer episode between male on foot (M) and female (F).

FIGURE 3. Four successive episodes in torch signaling with female; about 3 second gaps between episodes. Note that female fails to respond when the interval between the two flashes of the "male" is too short (Fig. 3b, c). (The female of Figs. 2 and 3 is the same as female No. 4 in Fig. 8).

the same male during 19 periods of unengaged flashing in flight, and 1.26 ± 0.02 sec in a second male during 12 cycles in flight during which attempts were being made to lure him to the torch (all at $27°$). The inconsequential differences indicate that the male's phrase-making is independent of visual input from the female.

The period of repetition of the phrase was 4.85 ± 0.013 sec in a total of 22 periods of two males in searching flight at $27°$. The question of whether the male's pacemaking rhythm is different during actual dialogue was not studied but no difference was noticed. However, when the male has alighted and is proceeding toward the female on foot, which happens during the final stages of courtship, the intervals between successive phrases are apt to be markedly irregular.

3. *Female's response delay*

At $27°$ two females in dialogue with males responded, respectively, after mean delays of 0.92 sec (2 cycles) and 0.94 ± 0.02 msec (range 0.86 to 1.1; 10 cycles), measured from the second of the male's paired flashes. These females and several
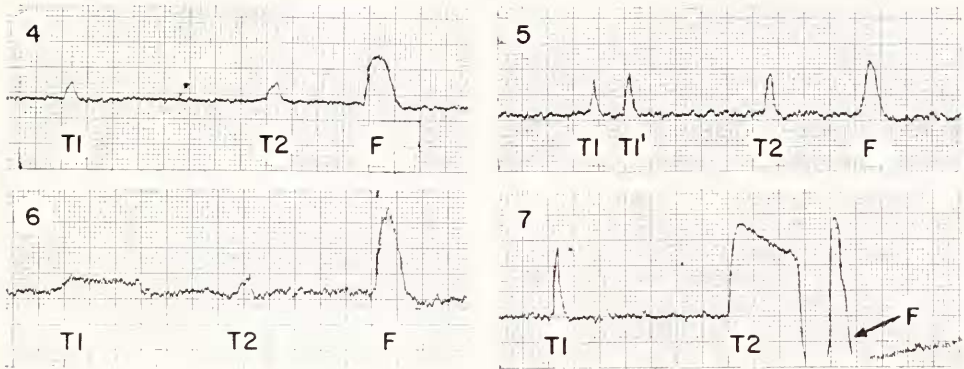
FIGURE 4. Response of female to paired flashes from torch.

FIGURE 5. Response of female to torch signals in which an extra flash (T'_1) is intercalated.

FIGURE 6. Response of female to torch signals in which the duration of T_1 is greatly increased.

FIGURE 7. Response of female in which the duration of T_2 is greatly increased. The dip in the photometer trace is an electrical artifact. The trace has been strengthened for reproduction. (The female of Figs. 4–7 is as female number 5 in Fig. 8).

others were tested with a total of 220 paired torch flashes. The delays recorded in the 127 responses are summarized in Table 1. This table shows that the responses of females to paired torch flashes involved the same order of delay as in dialogues with real males even though the intervals between the two torch flashes were in many instances well outside the range of intra-phrase intervals of actual males (extreme range among two males, 1.16 to 1.42 secs; among effective torch pairs 0.92 to 2.20 secs).

4. Intraphrase interval

The frequency distributions of the 127 responses and 93 non-responses to the 220 torch signals to the 6 females (Table 1) are plotted in Fig. 8. Granted that

TABLE I

Time relations during simulated courtship signaling. (T_1 = *first flash of simulated male phrase*; T_2 = *second flash*; F = *response of female*)

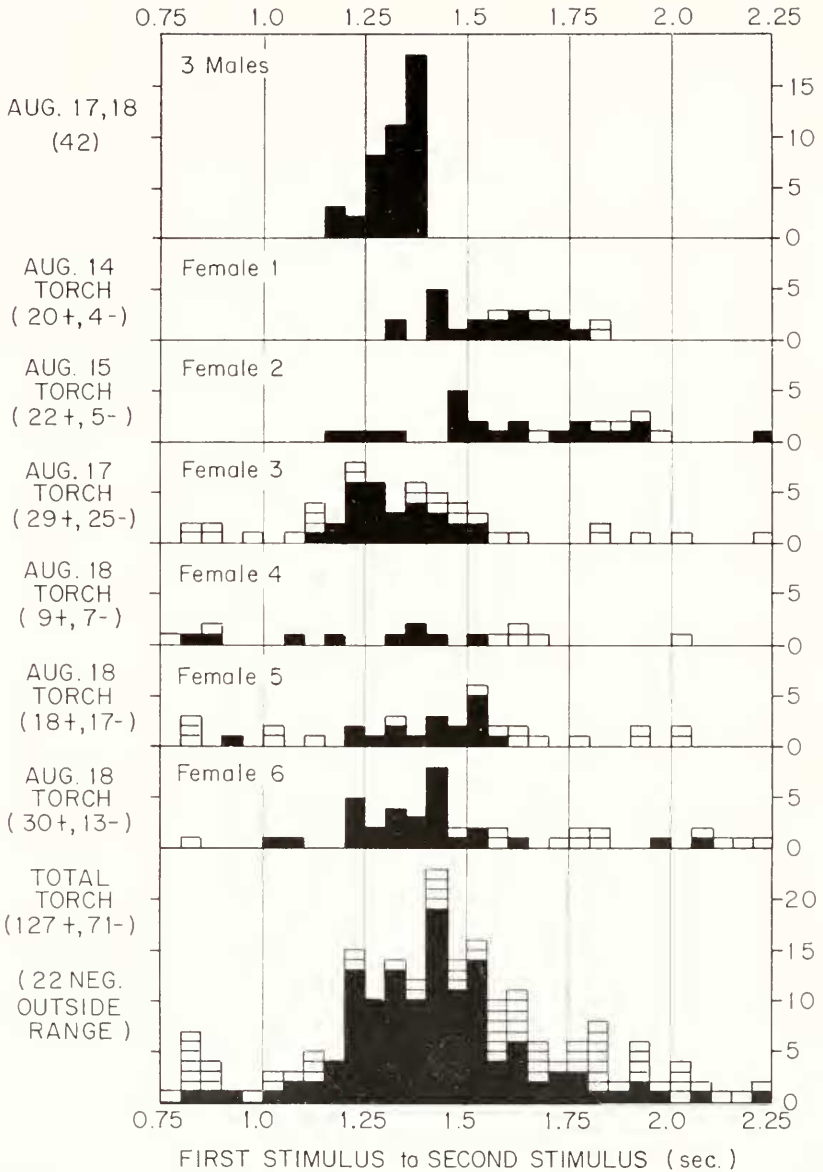| Date	T°	No. resp.	$T_1 - T_2$			$T_2 - F$			$T_1 - F$		
			M̄ S.E.	V		M̄ S.E.	V		M̄ S.E.	V	
VIII/14	(22°)	20	1.54 ± 0.03	0.09		0.96 ± 0.02	0.08		2.50 ± 0.03	0.06	
VIII/15	(27°)	21	1.58 ± 0.06	0.16		0.91 ± 0.02	0.08		2.47 ± 0.06	0.11	
VIII/17	(27°)	29	1.31 ± 0.02	0.08		0.94 ± 0.01	0.06		2.25 ± 0.02	0.06	
VIII/18	(27°)	18	1.37 ± 0.04	0.12		0.84 ± 0.02	0.08		2.21 ± 0.04	0.07	
VIII/18	(27°)	30	1.38 ± 0.04	0.15		0.80 ± 0.01	0.08		2.19 ± 0.04	0.10	
VIII/18	(27°)	9	1.21 ± 0.08	0.20		0.93 ± 0.04	0.11		2.14 ± 0.06	0.09	

FIGURE 8. Frequency distributions of natural phrase durations in three male fireflies (top line) and experimental torch phrases used in signaling to six different female fireflies (next six lines: last line shows total for torch signals). Black units indicate responses, white units are failures to respond.

failure to respond is much less significant than response (because a female presumably may fail to react by reason of the artificial flashes being too bright, too dim or too large in area, or for unknown reasons, as well as because of unacceptable

timing) and that response failure by the female is not rare during natural signaling in the field, it is apparent that the range of acceptable intra-phrase flash intervals is comparatively narrow. In five of the females the range within which a reasonable percentage of responses occurred was about 750 msec and in the sixth about 500 msec. Even so the range appears to be at least twice the actual spread of phrase intervals in male fireflies (top line, Fig. 8).

5. *Phrase format*

To explore the informational significance of the two flashes of the male's signal phrase each was modified separately in number and in duration in a number of torch simulation tests. T_2 proved much less labile than T_1, but disregarding failures of response these tests showed that either flash can be repeated at least once within a period of 250 to 500 msec or lengthened up to 820 msec without vitiating its role as an essential part of an effective stimulus to the female. Examples of such effects are given in Figures 5, 6 and 7.

As an additional probe of the criteria of acceptable stimulus the two flashes of the phrase were delivered by torch from opposite sides of the female. When the first of the stimulus pair was thus received mainly by one eye and the second by the other eye the female nevertheless responded normally in each of many trials.

6. *Male-male interactions*

On several occasions when a female was being stimulated repetitively by torch signals, flying males approached the scene, fell into step with the torch and entered into dialogue with the female. Except for the remote possibility that the flashing rhythm of these males just happened to be in the proper phase relation to the female's response to the torch at the time the male flew into range, such behavior indicates that the male recognizes not only the female's response delay interval but its relation to the preceding stimulus flashes.

7. *Starting point for female's response latency*

If the range of acceptable intra-phrase intervals could be made sufficiently large in proportion to the range of response latencies of the female it might be possible to deduce whether the female times her response from the first or from the second flash of the stimulus phrase. The argument would be that if the latency were timed from T_1, the T_1-T_2 interval should be inversely related to T_2-F whereas if timed from T_2 the two values should be independent. It would also be expected that T_1-F might be more variable than T_2-F if the latency is timed from T_2.

Figure 9 presents a scatter diagram for the 127 pairs of effective torch phrase intervals and the corresponding latencies for the six females (Table 1 data). The distribution is consistent with independence of T_1-T_2 and T_2-F, although the variability of T_2-F is great enough to weaken the conclusion. If the T_1-T_2 values are divided into two populations on the basis of being shorter than or longer than 1.4 seconds ($\overline{M} = 1.24 \pm 0.015$ for 60 phrases; $\overline{M} = 1.56 \pm 0.021$ for 67 phrases) the
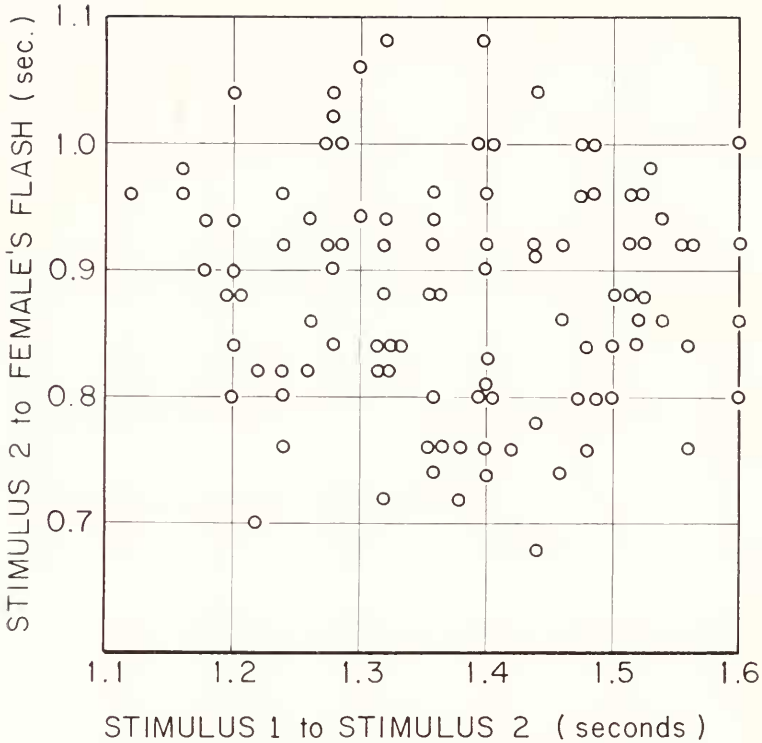
FIGURE 9.   Relationship between 127 paired torch phrases (abscissa) and corresponding
latencies of responses of females (ordinate) ; total for 6 dialogues.

corresponding T_2-F values are 0.89 ± 0.01 and 0.89 ± 0.01, again indicating independence of T_1-T_2 and T_2-F.   However, the fact that the coefficients of variation of T_1-F and T_2-F are not systematically different (Table 1) somewhat militates against distinguishing a starting point for the latency in this way.

DISCUSSION

Probably the most significant conclusion to be drawn from experimental signaling to females of *P. greeni* in the field is that response can be elicited over wide ranges of signal intensity, duration, source area, format and orientation providing only that the two flashes of the stimulus phrase are separated by the proper interval (roughly 1.1 to 1.7 secs at 27°).   This fact supports previous evidence of the primacy of time relations in firefly signaling systems but extends it to systems a step up in complexity from that of species like *P. pyralis,* where the proper stimulus to the female is a single flash.   In *P. greeni* the female must be able to recognize as an informational unit a pair of flashes separated by a particular range of intervals.   In this connection it is well to keep in mind that the female's response is a cue for the male to orient, not to flash, whereas the male's phrase is a cue to the female both to orient (her abdomen) and to flash.

Though these rough experiments in no sense define the female's receptor requirements, the pulse-duplication tests show that she becomes in effect refractory for several hundred milliseconds after stimulation. Thus, when the first flash of the male's phrase is doubled, the second element (T'_1, Fig. 5) does not supercede the initial flash (T_1) as the starting point of a response cycle nor does it take over the position of T_2 as time zero for the latency of the female's eventual response. Whether this refractoriness resides in the response mechanism or in the receptor part of the excitation system must remain moot at least until the flicker fusion frequency of the female's eye can be measured. The fact that the time of *ending* of a flash does not have any effect on the timing of the female's response even when the flash is long enough to have exceeded the ordinary limits of neural refractoriness (T_1, Fig. 6; T_2, Fig. 7), suggests that only an *increase* in light intensity can qualify as a stimulus. Such a conclusion would be consistent with work on signaling in *Luciola lusitanica* in which Papi (1969) demonstrated the importance of the rate of rise of light intensity during the stimulus flash.

The experiments in which the two flashes of the phrase were delivered alternately from opposite sides of the female argue that the mechanism that is successively activated by the two events is, as expected, central, not peripheral and that the dialogue does not require a special relative orientation of male and female.

The fact that the female's latency is unchanged with changing stimulus pulse duration again argues that the effective part of the flash is the initial rise in light intensity—*i.e.,* that the female's response is an "on" response.

The fact that the female accepts a considerably wider range of intraphrase intervals than is normally provided by actual males (Fig. 8) is in line with Lloyd's suggestion (personal communication) that a workable code, beside being exclusive of those of other species of firefly active at the same time, must allow for the likelihood that a male in flight may have a considerably different body temperature from that of a female perched near the ground.

Durations of interflash intervals in different species of fireflies in different localities and of fireflies studied by different investigators should be compared with caution, particularly when measured at nominally different temperatures. However, it appears that the interflash interval in the male's stimulus phrase in the Cape Cod *P. greeni* agrees with that of the Florida form in being distinct from Lloyd's figures for the Florida strains of *P. consanguineus* (*ca.* 0.5 sec at 26°) and *P. macdermotti* (2 secs at 23°) and the Maryland strain of *macdermotti* (1.8 sec at 24°). Our mean value of about 1.3 sec at 27° seems disturbingly different from Lloyd's Florida value of 1.2 sec at about 19°, but our response latency for the female (*ca.* 0.9 sec at 27°) is quite close to Lloyd's figure of 1 sec at 24°. Though the temperature is not given, Carlson's (1969) data for laboratory tests indicate that his Long Island species could also be *P. greeni*. Thus the validity of *P. greeni* as a separate species seems to be supported, as is the mutual independence of *greeni, macermotti* and *consanguineus: i.e.,* the males' phrases are probably sufficiently different to be distinguishable by the respective females.

The ability of a male photinid to recognize that a courtship exchange between a female and another male (or torch) is underway and to shift his flashing rhythm so as to coincide with that of the engaged male, was first noticed among coteries of *P. pyralis* males in natural courtship (Buck, 1935) and confirmed in torch

exchanges in the same species (Buck and Buck, 1968). Lloyd (1971) has illustrated an interesting instance of similar ability in *P. greeni:* A male, after having seen an artificial two pulse pattern and the female's response, was able to supply the second flash of a stimulus phrase in which the first flash was artificial light. Male-male synchronization has recently been analyzed in a New Guinea *Pteroptyx* (Hanson, Case, Buck and Buck, 1971) and appears to be quite different from that of the courtship signals. In any case the coexistence of two such well-developed communication systems in one species of *Photinus* seems remarkable.

The question of the takeoff point for the female's response latency is of sufficient interest neurologically that it would be desirable to have more conclusive statistical evidence of the independence of T_1-T_2 and T_2-F. More T_1-T_2 intervals at the extremes of the acceptable response range are needed, a desideratum requiring more precise and reproducible signal control than possible with a manual pushbutton. However, an additional indication that the female times her response from T_2 is provided by a few instances in which the female responded to the first of the two flashes of the phrase. In such instances the latency measured was in the usual T_2-F range.

SUMMARY

1. In duplicating the courtship signals of *Photinus greeni* it was found possible to substitute flashes of artificial light for either the male or the female.

2. In confirmation of previous work, the courtship signals of *P. greeni* were found to depend on the female's recognition of the male's stimulus signal of two flashes and the male's recognition of the female's fixed response interval after his second flash. At 27° C the acceptance limits of the male's phrase were from about 1.1 to 1.7 seconds and the limits of the female's response latency were from about 0.8 to 1.1 seconds.

3. Within wide limits the delivery rate of the male's two-flash signal ("phrase"), and the intensities, durations and source areas of the flashes of male and female, are immaterial to the success of the signaling.

4. The two flashes of the male's stimulus phrase can be made multiple, or increased several-fold in duration, without preventing the female from responding, as long as the proper time relation between the starts of the first flash and one subsequent one is preserved. From this it is concluded that it is the rise in light intensity that is perceived by the female and that she is refractory to new visual input for several hundred milliseconds after being stimulated.

5. Instances in which males fell into step with torch flashes being used to stimulate females serially are interpreted as male-male communication.

6. It is concluded tentatively that the female times her response latency from the second flash of the male's stimulus phrase.

7. The behavioristic data for the Cape Cod *P. greeni* are consistent with its belonging to the same species as the Florida strain and with being distinct from *P. consanguineus* and *P. macdermotti.*

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